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The final publication is available at:

<https://doi.org/10.1002/jsfa.9031>

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**Evaluation of postharvest calcium treatment and biopreservation with
Lactobacillus rhamnosus GG on the quality of fresh-cut 'Conference' pears**

Running title: Evaluation of the effect of calcium and probiotic bacteria on quality of fresh-cut pears

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ABSTRACT

BACKGROUND: Biological preservation with probiotic bacteria has arisen as an alternative to control the growth of foodborne pathogens on food. The objective of this work was to evaluate the effect of postharvest calcium application and biopreservation with *Lactobacillus rhamnosus* GG on the quality and bioaccessibility of total phenolic content and antioxidant activity in fresh-cut pears.

RESULTS: The immersion of whole pears in a calcium chloride solution did not provide added value. Despite the increase in observed activity of PME and PPO enzymes in fresh-cut pears during storage, the browning index and firmness values were constant for all samples. The antioxidant properties, including antioxidant activity, total phenolic content and vitamin C content, were maintained during storage, but a significant decrease was observed after *in vitro* simulated digestion. Ca/LGG samples showed the lowest calcium content (1.75 ± 0.00 g kg⁻¹) after 9 d of storage at 4 °C. In general, the overall visual quality scores were higher in fresh-cut

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/jsfa.9031

pears treated with *L. rhamnosus* GG than in non-treated pears, with the highest values in the NoCa/LGG (7.7 ± 0.2) samples after 9 d at 4 °C.

CONCLUSION: Fresh-cut pears with a postharvest treatment of calcium and immersed in a solution containing antioxidant agents and probiotic bacteria could be a suitable alternative to dairy products for maintaining the overall quality of fruit for up to 9 days of storage.

Keywords: probiotic; *Lactobacillus*; pear; quality; digestion.

ABBREVIATIONS

1-MCP: 1-methylcyclopropene

AA: Antioxidant activity

BI: Browning Index

CK: Control

CMC: Carboxymethyl cellulose

L. rhamnosus GG (manuscript): *Lactobacillus rhamnosus* GG

LGG (figures and tables): *Lactobacillus rhamnosus* GG

NAC: N-acetylcysteine

PG: Polygalacturonase

PME: Pectinmethyl esterase

POD: Peroxidase

PPO: Polyphenol oxidase

TPC: Total Phenolic Content

INTRODUCTION

Pears (*Pyrus communis* L.) have a low protein, lipid and glucose content and are rich in other sugars such as fructose, sorbitol, and sucrose. Pears also contain micronutrients such as vitamins, minerals, and antioxidants¹. The annual consumption of pears in Spain, which is 5.44 kg per capita, decreased by 0.8 % in 2016². In 2015, 355,410 tons of pears were produced in Spain, 39.5 % of which were produced in Lleida³.

In recent years, the minimally processed food market has increased its presence in the food industry⁴ due to societal changes in lifestyle. The principal attraction of fresh-cut fruit and vegetables is their convenience and quality⁵. In Europe, fresh-cut fruit constitutes approximately 10 % of the total fresh-cut market volume, and in countries such as Germany and Spain, the market has grown more than in other western countries⁶. Fresh-cut fruit can be defined as any fresh fruit that has been subjected to peeling, trimming, or washing and cutting⁷. Fresh-cut fruit are more perishable than fresh whole fruit because in minimal processing, the natural skin barrier is removed, and microbial invasion, respiration, ethylene production, water loss and microbial decay are promoted⁸. These factors can reduce the visual quality (e.g., colour, texture, flavour) and nutritional properties, decreasing the shelf-life of fresh-cut fruit.

Calcium is an essential nutrient in fruit and vegetables. Calcium deficiency has been implicated in several disorders such as bitter pit in apples and pears^{9, 10}, brown rot in apples¹¹ or brown spots in pears¹². Calcium plays an important role in the maintenance of firmness¹³ due to its ability to activate pectin methyl esterase (PME, EC 3. 1. 1. 11)¹⁴ and at the same time to cross-link with the carboxyl groups of pectin found in the middle lamella¹⁵. In fresh-cut fruit, calcium is usually applied by dipping the fruit in a solution that contains ascorbic acid, calcium salt and an organic acid¹⁶.

Natural preservation using biological agents, such as probiotics, has been singled out as an emerging technology¹⁷. A probiotic has been defined as containing “live microorganisms which when administered in adequate amounts confer a health benefit on the host”¹⁸. Probiotics

should not only survive in the food product but should also be able to reach the small intestine alive¹⁹. Most common probiotics belong to the heterogeneous group of lactic acid bacteria (e.g., *Lactobacillus*, *Enterococcus*) and to the genus *Bifidobacterium*^{20, 21}. *Lactobacillus rhamnosus* GG is an extensively studied strain with well-documented probiotic properties¹⁹. Iglesias et al.²² reported that the presence of *Lactobacillus rhamnosus* GG in fresh-cut pears significantly reduced the growth of *L. monocytogenes* and its adhesive properties and ability to invade Caco-2 cells.

Moreover, fresh-cut fruit and vegetables enriched with probiotic bacteria could be an alternative for people who are allergic to animal proteins or are lactose intolerant, hypercholesterolaemic or vegetarian²²⁻²⁴. In previous studies, the use of microorganisms from the genus *Lactobacillus* as a probiotic in fresh-cut jackfruit¹⁷, pineapple^{25, 26} and apples^{4, 19} had no notable adverse effects on the physicochemical and sensory qualities of those products.

It has been demonstrated that *Lactobacillus rhamnosus* GG on fresh-cut pears was able to survive under *in vitro* simulated gastrointestinal conditions²². Nevertheless, it is essential to know how digestion affects the bioactive compounds and their stability and consequently their bioaccessibility²⁷. Bioaccessibility is defined as the quantity of compounds that are released from the food matrix and presented to the intestinal brush border for transport into the cell^{28, 29}. *In vitro* digestion is often used to simulate gastrointestinal conditions due to the safety and ethical restrictions of *in vivo* models^{30, 31}.

As previously reported by Iglesias et al.³², the postharvest calcium treatment and application of *L. rhamnosus* GG had no effect on either the respiration rate, soluble solids content, titratable acidity or the ethanol and acetaldehyde concentration of fresh-cut pears. The *L. rhamnosus* GG population was positively correlated with higher emissions of certain esters and alcohols. Moreover, the population of *L. monocytogenes* inoculated with *L. rhamnosus* GG decreased 1.8-log units after 9 d of storage.

The objective of the present study was to evaluate the effect of postharvest treatment with calcium and the probiotic bacteria *Lactobacillus rhamnosus* GG on parameters of physical and chemical quality, enzymatic activities (polyphenol oxidase [PPO, EC 1. 10. 3. 1], peroxidase [POD, EC 1. 11. 1. x], polygalacturonase [PG, EC 3. 2. 1. 15], and pectin methyl esterase), vitamin C content, antioxidant activity and calcium content of minimally processed pears during their shelf-life. A study of simulated *in vitro* gastrointestinal digestion was also conducted to evaluate the bioaccessibility of total phenolic content and antioxidant activity at phases of digestion.

EXPERIMENTAL METHODS

Plant material and postharvest treatment

‘Conference’ pears were obtained from a local farmer (Grealó, Lleida) at commercial maturity (62.6 N, 12.6 % soluble solids, 2.46 g malic acid L⁻¹). Fruits free of visual defects and uniform in colour and size were selected and divided into two lots. Postharvest treatments consisted of fruit dips at 25 °C for 10 min in the following solutions: water (control) and 1 % CaCl₂. Fruits were then allowed to dry at room temperature before storage at temperatures ranging from 0 to -0.5 °C over 8 months under controlled atmospheric conditions (2 % O₂ and 1 % CO₂) and 85 % RH.

Bacterial strain

The antagonist used in this study was the probiotic strain *Lactobacillus rhamnosus* GG (obtained from Ashtown Food Research Centre, Teagasc, Ashtown, Dublin, Ireland). The growth conditions and final concentration on fresh-cut pears were previously reported by Iglesias et al.³², and the viability of the probiotic strain on fresh-cut pears was monitored during storage (0, 2, 6 and 9 days) in that study.

Processing, packaging and storage

Pears were cleaned, sanitized by immersion into a 0.1 g L⁻¹ NaOCl solution adjusted to pH 6.5 using citric acid, rinsed and dried prior to cutting operations. Pears were peeled and cut into 10 wedges using a handheld apple corer and slicer. The cut fruit were then dipped into an ice bath. Untreated pears and pears treated postharvest with calcium (1 % CaCl₂) were divided into 2 batches each. Each batch was dipped (1:2 w/v) for 2 min at 150 rpm (horizontal shaker) in a cold water and antioxidant solution (2 % ascorbic acid, 2 % sodium citrate and 1 % calcium chloride). Two of these batches were treated with *Lactobacillus rhamnosus* GG (10⁸ CFU mL⁻¹ in bath; 4 x 10⁷ CFU g⁻¹ on fresh-cut fruit) and the results were compared to a negative control (without the *Lactobacillus* treatment). Treated fruit were allowed to dry under ambient

conditions. Approximately 120 g of cut pears were placed on 375 mL polypropylene trays (Anguera, Spain) and heat-sealed with a non-peelable polypropylene plastic film (PP-110, ILPRA, Italy) of 64 μm thickness with an O_2 permeability of $110 \text{ cm}^3 \text{ m}^{-2} \text{ d}^{-1} \text{ atm}^{-1}$ at 23°C and 90 % RH³². Afterwards, the pear trays were stored at 4°C for 9 d. At days 0, 2, 6, and 9, a portion of each sample was frozen with liquid nitrogen, pulverized and stored at -80°C for enzymatic assays, vitamin C analysis, phenolic content analysis, and antioxidant activity and calcium determination.

Physical and chemical assays

Physical (colour and firmness) and chemical (titratable acidity and soluble solids content) assays were performed according to methods described by Plaza et al.³³. The soluble solids content (SSC) was expressed as a percent and the titratable acidity (TA) was expressed as grams of malic acid L^{-1} . Those determinations were performed on whole pears. Three determinations were performed for each treatment. The firmness evaluation was conducted by determining the maximum strength necessary for a cylindrical probe of 4 mm in diameter to penetrate 10 mm into a pear sample using a TA-TX2 Texture Analyser (Stable Micro Systems Ltd., Surrey, England). Colour was measured on the peel of the whole pear and the flesh of fresh-cut pears with a CR-200 Minolta Chroma Meter (Minolta, Inc., Tokyo, Japan) with a D65 illuminant and a 10° observer angle. The values a^* , b^* and L^* were used to calculate the browning index (BI), as previously described by Liu et al.³⁴.

Assessment of overall visual quality

The assessment was conducted as described by Altisent et al.³⁵. The visual quality was assessed by an untrained panel based on the following hedonic scale: 9 = excellent; 7 = very good; 5 = good, at the limit of marketability; 3 = fair, at the limit of usability; and 1 = poor, inedible.

Enzymatic assays

PPO and POD activity

The PPO and POD activities were measured as described by Plaza et al.³³. A PPO unit was defined as 0.01 absorbance units per minute, and PPO activity was defined as the change in absorbance per second per kilogram on a fresh weight basis. A POD unit was defined as 0.01 absorbance units per minute, and POD activity was defined as the change in absorbance per second per kilogram on a fresh weight basis.

PME and PG activity

PME activity was determined according to the method of Plaza et al.³⁶ with some modifications. Briefly, the enzyme was extracted by the homogenization of 5 g of frozen sample with 10 mL of an extraction solution (1 mol L⁻¹ NaCl in 0.2 mol L⁻¹ sodium phosphate buffer, pH 7.5). The resulting mixture was shaken for 10 min at 4 °C, centrifuged at 13523 × g for 20 min at 4 °C and then filtered. The supernatant was then collected and stored at -80 °C until the determination of enzyme activity. PME activity was measured titrimetrically at 25 °C. Briefly, 2 mL of the enzymatic extract were mixed with 40 mL of a pectin-salt substrate solution (0.35 % pectin and 0.1 mol L⁻¹ NaCl). The solution was adjusted to pH 7.5 with 1 mol L⁻¹ NaOH. After the pH reached 7.5, 0.1 mL of 0.05 mol L⁻¹ sodium hydroxide was added. The time elapsed until the pH of the solution regained pH 7.5 was measured. A PME unit was defined on a fresh weight basis as the amount of enzyme required to release 1 mol of carboxyl groups per second.

PG activity was determined according to the method of Van linden et al.³⁷ with some modifications. Six grams of frozen sample were homogenized with 9 mL of cold deionized water. The mixture was adjusted to pH 3.0 using HCl, stirred for 5 min and centrifuged at 13523 x g for 20 min at 4 °C. The pellet was recovered and resuspended in 6 mL of cold 1.2 mol L⁻¹ NaCl, the solution was adjusted to pH 6.0 and stirred for 5 min and then centrifuged at 13523 x g for 20 min at 4 °C. Finally, the collected extract was stored at -80 °C until PG determination.

Reaction mixtures consisted of 350 μL of a 0.2 % (w/v) buffered PGA solution containing 0.04 mol L^{-1} Na acetate at pH 4.4 and 50 μL of PG extract. The reaction mixtures were then incubated at 40 $^{\circ}\text{C}$ for 10 min. Two millilitres of cold borate buffer (pH 9.0, 0.1 mol L^{-1}) were added to each sample to stop the reaction, and 0.4 mL of a 1 % 2-cyano-acetamide solution were added. The mixture was incubated for 10 min at 100 $^{\circ}\text{C}$ and then immediately cooled in an ice bath. The absorbance was determined using a Genesys 10S UV-Vis spectrophotometer (Thermo ScientificTM, Spain) at 276 nm and 22 $^{\circ}\text{C}$. A standard calibration curve was generated at 276 nm using known concentrations of galacturonic acid (ranging from 0 to 3 x 10⁻³ mol L^{-1} final volume). The enzyme activity was calculated on a fresh weight basis as the release of reducing groups per second.

Vitamin C analysis

The assay was carried out as described by Altisent et al.³⁵. The reduction of dehydroascorbic acid to ascorbic acid was employed to determine the total vitamin C content using TCEP as the reducing agent. The total vitamin C content was determined using high-performance liquid chromatography (HPLC). The results were expressed on a fresh weight basis as grams of ascorbic acid per kilogram.

Determination of total phenolic content

The total phenolic content was determined using the Folin Ciocalteu method³⁸, according to the modifications described by Altisent et al.³⁵. The results were expressed on a fresh weight basis as grams of gallic acid equivalent per kilogram.

Determination of antioxidant activity

Antioxidant activity (AA) was determined by two different methods: a 2,2-diphenyl-1-picrylhydrazyl (DPPH[•]) radical scavenging assay and a ferric reducing antioxidant power (FRAP) assay. The extraction and assays were conducted according to the methods described by

Plaza et al.³³. The results were expressed on a fresh weight basis as moles of ascorbic acid equivalents per kilogram.

Determination of calcium

The determination of calcium was performed using a HORIBA Jobin Yvon inductively coupled plasma optical emission spectrometer (ICP-OES), Model Activa, equipped with a Meinhard nebulizer. The instrument was calibrated using a mono-elemental atomic absorption standard solution for calcium (1000 mg L⁻¹). The sample pretreatment (digestion procedure) was conducted in an MLS 1200 mega microwave (Milestone, Milan, Italy) with HNO₃ (700 mL L⁻¹), H₂O₂ (300 mL L⁻¹) and nanopure water. The optic emission was measured at 393 nm. The results were expressed on a fresh weight basis as grams of calcium per kilogram.

***In vitro* simulated gastrointestinal digestion**

In vitro simulated gastrointestinal digestion was performed according to the method described by Minekus et al.³⁹ with minor modifications³¹. The digestion was performed in triplicate for each sample at each sampling time. A blank was prepared using only distilled water in place of the sample following the same procedure. The results were compared with undigested samples. Antioxidant activity (FRAP and DPPH[•]) and total phenolic content were determined after the gastric and intestinal phases. The percentage of loss was calculated using Eq. (1).

$$Loss (\%) = \frac{\text{undigested values} - \text{intestinal values}}{\text{undigested values}} \times 100 \quad (1)$$

Statistical analysis

The results were expressed as the mean \pm standard deviation of three independent determinations. Differences among treatments were evaluated by using a one-way analysis of variance (ANOVA) and were considered to be significant at $P < 0.05$ (95 % confidence level). A

Tukey's test and t -tests were used to determine the differences between means. JMP 8 software was used to conduct the statistical analyses (SAS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

Physical and chemical quality parameters and overall visual quality

The quality parameters of whole pears after 8 months of storage are shown in Table 1. In general, the postharvest calcium treatment did not affect overall quality, with the exception of acidity. Untreated pears showed higher acidity (2.20 ± 0.12 g malic acid L⁻¹) than treated pears (1.73 ± 0.18 g malic acid L⁻¹). In contrast, Zhao & Wang⁴⁰ demonstrated that dipping apples in a solution of 5 % CaCl₂ for 10 min moderated the decrease in titratable acidity values. Liu et al.⁴¹ showed that apricots treated with 1 % or 3 % CaCl₂ for 2 min and stored at 5 °C had a higher TA than the control, and the decrease in TA was slowed. They suggested that low temperature storage combined with calcium treatment could reduce acid oxidation.

The results of the physicochemical parameters of fresh-cut pears are shown in Table 2. Overall, neither calcium chloride nor *L. rhamnosus* GG affected the firmness of fresh-cut pears, and there were no significant differences ($P>0.05$) over the storage period for each treatment. In general, BI values were maintained during the storage period for all samples except for samples with postharvest calcium treatment but without probiotic bacteria. There were no significant differences ($P>0.05$) between samples until day 6 of storage at 4 °C.

Overall, samples treated with *L. rhamnosus* GG had higher visual quality scores than non-treated samples (Fig. 1), and only the NoCa/LGG samples were still marketable (7.7 ± 0.2) after 9 days of storage at 4 °C.

Enzymatic activity

Polyphenol oxidase (PPO) and peroxidase (POD) assays

The postharvest application of 1 % CaCl₂ resulted in an increase in PPO and POD activities in whole pears (Table 1). In general, PPO activity increased in fresh-cut pears with postharvest calcium treatment after 9 day of storage at 4 °C (Table 2). Despite the increase in PPO activity, a non-significant change in BI was observed. Koushesh Saba & Sogvar⁴² reported

that PPO enzyme activity increased in all fresh-cut apples during the storage period irrespective of the treatment applied (T₁: control, T₂: CMC + 0.5 % CaCl₂, T₃: CMC + 0.5 % CaCl₂ + Ascorbic Acid). López-López et al.¹⁶ demonstrated a rapid increase in PPO activity during the first 15 d of storage for apple slices treated with NAC (0.05 mol L⁻¹), CaCl₂ (0.5 %), or a combination of NAC-CaCl₂. PPO has been considered the primary enzyme involved in the loss of quality in fresh-cut fruit because its activity is related to enzymatic browning⁴³.

The effect of PPO activity on fresh-cut pear browning is a controversial issue. Gomes et al.⁴⁴ reported that PPO activity was not the best predictor of tissue browning in fresh-cut ‘Rocha’ pears, either in the presence or in the absence of inhibitors. However, Soliva-Fortuny et al.⁴⁵ reported that the browning of fresh-cut ‘Conference’ pears could be correlated with PPO values, and they suggested that browning reactions are not only influenced by the presence of PPO but also by the availability of oxygen and the presence of phenolic substrates. In the present study, fresh-cut pears were immersed in an antioxidant solution (2 % ascorbic acid, 2 % sodium citrate and 1 % calcium chloride) to avoid browning and softening. Overall, the browning index remained constant over the storage period, whereas PPO activity increased. Oms-Oliu et al.⁴⁶ suggested that a high concentration of ascorbic acid could diffuse into fresh-cut pear tissue and lead to cell disruption and the subsequent decompartmentalisation of enzymes.

In general, POD activity was maintained after 9 days of storage at 4 °C for samples treated with *L. rhamnosus* GG (Table 2). No significant differences (P>0.05) in POD activity were observed for different samples at the end of the storage period. In contrast, Xu et al.⁴⁷ reported that POD activity increased more in whole litchi fruit treated with *Lactobacillus plantarum* (10⁸-10⁹ CFU mL⁻¹ for 2 min) than in untreated samples after storage. Consistent with these observations, Konappa et al.⁴⁸ observed that tomato seedlings treated with lactic acid bacteria (10⁸ CFU mL⁻¹) also showed higher POD activity than the untreated samples.

Pectinmethyl esterase (PME) and polygalacturonase (PG) assays

The postharvest treatment with CaCl_2 produced a significant increase ($P < 0.05$) in the PG activity of whole pears, whereas no effect was observed on PME activity (Table 1). Despite the observed increase in PG activity, no changes in the firmness of the samples were detected. Belge et al.⁴⁹ demonstrated that PG activity increases in sweet cherries treated with 1.5 and 3 % CaCl_2 after a 15-day storage period with no apparent relationship to firmness. Similarly, Manganaris et al.⁵⁰ indicated that PG activity was higher in peaches treated with calcium chloride (62.5 and 187.5 mmol L^{-1}) compared with untreated fruit after 21 d under cold storage (5 °C). Manganaris et al.⁵¹ detected an increase in exo-PG and endo-PG activities in nectarines treated with calcium lactate or calcium propionate at a high concentration (7.5 g L^{-1}), and they suggested that this might be due to surface injury. The action of PG is modified by the presence of calcium in the cell wall⁵². PG activity is greatly inhibited by high calcium concentrations, and in calcium-deficient tissues, PG activity is increased⁵³. Recently, Fortes et al.⁵⁴ suggested that exogenous calcium inhibits PG activity and that a reduction in calcium concentration could be linked to an increase in PG activity.

In general, PME activity increased in fresh-cut fruits after 9 days of storage at 4 °C (Table 2). However, there were no significant differences ($P > 0.05$) in PME activity among samples at the end of the storage period (9 d). Similarly, PG activity was maintained during the storage period for all fresh-cut samples without a significant observed effect ($P > 0.05$) of the addition of *L. rhamnosus* GG (Table 2). The maintenance of PG activity could be related to the preservation of firmness that was observed in all samples. The dipping treatment used in the fresh-cut processing contained calcium chloride, and it is known that calcium can have inhibitory effects on PG activity by solubilizing PG-mediated hydrolysis products⁵⁵.

Vitamin C

There were no significant differences ($P > 0.05$) in the vitamin C values of whole pears regardless of the postharvest calcium treatment (Table 1). In fresh-cut pears (Table 3), the initial

values of vitamin C were very high in response to the antioxidant dipping treatment containing 2 % ascorbic acid. Moreover, the vitamin C content significantly decreased during shelf-life, resulting in values of approximately 0.01 g kg^{-1} after 9 d of storage. This behaviour is consistent with other studies on fresh-cut fruits. For instance, Martins et al.⁵⁶ observed that fruit salads immersed in 1 % ascorbic acid and *Lactobacillus rhamnosus* HN001 showed high initial values of vitamin C followed by a marked reduction after a 5-day storage period. Similarly, Cocci et al.⁵⁷ reported that fresh-cut apples dipped in 1 % ascorbic acid and 1 % citric acid showed initial values that were higher than non-treated fruit due to the anti-browning treatment. However, they observed a decrease of up to 80 % after 1 d of storage with residual levels maintained until day 6. Ascorbic acid is typically located in the vacuole of the cell and is protected from oxidation by low pH and phenolic flavonoids^{57,58}. However, exogenous ascorbic acid is probably housed in a different location and is therefore more exposed to O_2 ⁵⁷, enzyme ascorbate oxidase or oxidizing enzymes such as POD⁵⁶.

No clear influence of *L. rhamnosus* GG on vitamin C content was observed (Table 3). The control samples (NoCa/NoLGG) showed the lowest vitamin C content ($0.99 \pm 0.07 \times 10^{-2} \text{ g kg}^{-1}$) after 9 days of storage at 4 °C, and there were no significant differences ($P>0.05$) between the other samples. Russo et al.²⁶ also observed that there were no significant differences ($P>0.05$) in vitamin C content between fresh-cut pineapples treated with probiotic bacteria (*Lactobacillus plantarum* B2 or *Lactobacillus fermentum* PBCC11.5) and the control after an 8 day storage period. However, Russo et al.²⁴ reported that the treatment with *Lactobacillus plantarum* B2 ($5 \times 10^9 \text{ CFU mL}^{-1}$) resulted in a decrease in ascorbic acid content in fresh-cut melon.

Total phenolic content and antioxidant activity

There were no significant differences ($P>0.05$) in TPC or AA of whole pears after 8 months of storage (Table 1). Zhao & Wang⁴⁰ also reported that there were no significant differences ($P>0.05$) in TPC between whole apples dipped in a solution containing 5 % CaCl_2 and untreated samples after 28 d of storage (20 °C). Nevertheless, Kou et al.¹² observed that

‘Huangguan’ pears treated with 2 % CaCl_2 showed higher values of phenolic compounds than untreated samples after 8 months of storage at 0 °C. Ayón-Reyna et al.⁵⁹ showed that the TPC was significantly higher in papayas with a hydrothermal-calcium chloride treatment (1 % w/v) than in the control after 20 d at 12 °C. Moreover, Javed et al.¹³ indicated that guava fruit dipped in an aqueous solution of calcium lactate (1-3 % w/v) for 5 min showed a higher TPC and AA than the control samples after 24 d at 10 ± 1 °C. As Kou et al.¹² have suggested, the high values of TPC could be linked to the inhibition of PPO activity. PPO is a copper-containing enzyme and could be activated with the help of Cu^{2+} . Hence, the inhibition of PPO by a calcium application might be due to an impact on Cu^{2+} .

Neither the postharvest calcium treatment nor the probiotic application was observed to influence the TPC or AA of fresh-cut pears during storage (Table 3). Russo et al.²⁴ reported that probiotic bacteria had no effect on the TPC and AA of fresh-cut cantaloupe, and those parameters remained stable during storage. Rößle et al.⁶⁰ reported that apples slices treated with probiotics showed a loss of TPC after 5 d, suggesting that treatment with a probiotic solution had little effect on the loss of polyphenols. Consistent with this, Russo et al.²⁶ showed that treatment with a probiotic solution did not significantly affect the TPC or AA values of fresh-cut pineapples.

In general, the TPC remained unchanged during 9 d of storage, whereas a decrease in the AA was observed in all samples irrespective of the treatment applied. This trend could be related to the addition of calcium chloride and ascorbic acid during the fresh-cut process. Koushesh Saba & Sogvar⁴² reported that the TPC decreased and the AA was maintained in fresh-cut apples coated with CMC containing calcium chloride (0.5 %) after 12 d of storage at 4 °C. Aguayo et al.⁶¹ showed that the TPC and AA (DPPH[•] and FRAP) decreased after 28 d of storage at 4 °C in fresh-cut apples treated with a 6 % calcium ascorbate solution. Bico et al.⁶² observed that the TPC of fresh-cut bananas dipped in a 1 % (w/v) calcium chloride solution for 3 min decreased after 5 d of storage at 5 °C. In the study conducted by Aguayo et al.⁶³, the TPC decreased and AA was maintained in fresh-cut apples treated with a 2 % calcium ascorbate and

stored for 7 d at 4 °C. The absence of changes in TPC values relative to AA values suggests that the level of phenolics could be controlled by mechanisms other than ascorbic acid⁶³.

Calcium content

The postharvest calcium application did not induce a change in the calcium levels in comparison with non-treated whole pears after 8 months of storage (Table 1). However, Kou et al.¹² showed that the postharvest application of 2 % CaCl₂ increased the concentration of calcium in peeled ‘Huangguan’ pears after 8 months of storage. Similarly, Gago et al.¹⁰ reported that ‘Golden Delicious’ apples treated with CaCl₂ (1.5 % w/v) and 1-MCP showed higher calcium concentrations than the control fruit after 180 d of storage. Moreover, in the studies conducted by Manganaris et al.^{50, 51}, peaches and nectarines treated with three different calcium solutions (chloride, lactate or propionate) showed a higher calcium content than the control after 28 and 42 d of cold storage, respectively. Beirão-da-Costa et al.¹⁵ reported that the application of calcium to kiwifruit by immersion of the whole fruit in solutions of three different calcium chloride concentrations (1 %, 2 % and 3 %, w/v) combined with heat treatment (45 °C) prevented the diffusion of calcium into the fruit and instead lead to some loss of calcium ions.

In general, the calcium content of fresh-cut pears was higher than on whole pears due to treatment with the antioxidant solution, which contained 1 % CaCl₂ (Table 3). Moreover, calcium levels were maintained until the end of the storage period. Samples treated with calcium and *L. rhamnosus* GG showed the lowest values (1.75 ± 0.00 g kg⁻¹) after 9 d of storage at 4 °C. Different studies that have focused on the application of probiotic bacteria^{20, 21, 24, 26, 64}, particularly *Lactobacillus rhamnosus*^{4, 22, 23, 56, 60, 65–67}, to fruit did not study the possible use of calcium by probiotic bacteria as a nutrient or as a mechanism of action against pathogenic bacteria.

In vitro simulated gastrointestinal (GI) digestion

The GI simulation of whole and fresh-cut pears was conducted at two sampling times: 0 and 9 days of storage. Iglesias et al.²² conducted an experiment where the viability of *L.*

rhamnosus GG in fresh-cut pears was studied under similar gastrointestinal conditions. It was observed that *L. rhamnosus* GG (inoculum of 10^{10} CFU mL⁻¹) was able to survive the GI phase, and viability was maintained up to 10^8 CFU g⁻¹ in pear wedges. Moreover, high adhesion (80 %) of *L. rhamnosus* GG was observed. In general, in the present study, the AA and TPC of fresh-cut pears decreased dramatically after GI digestion.

Antioxidant activity

The DPPH[•] and FRAP values of digested whole and fresh-cut pears are shown in Fig. 2. Total AA in both gastric and intestinal digests were lower than those obtained from chemical extraction (undigested). Zudaire et al.³¹ suggested that the antioxidant components present in fruit and vegetables could be potentially unstable due to pH changes and enzymatic degradation.

The decrease in AA was approximately 86-90 % in whole pears and 61-96 % in fresh-cut pears in comparison with the undigested samples. Schulz et al.²⁹ suggested that this decrease could be due to a decrease in the TPC and/or transformation into different structural forms with other chemical properties. In fresh-cut pears, the decrease in AA was greater at day 0 (approximately 95 %) than at day 9 (approximately 80 %) due to differences in the initial values. However, several studies reported that the DPPH[•] values of some fruit and fruit and vegetable juices decreased after the intestinal phase of digestion⁶⁸⁻⁷¹. Bouayed et al.⁷² also observed that FRAP antioxidant activity decreased in 4 apple varieties after gastrointestinal digestion. However, Wootton-Beard et al.⁷⁰ reported that the FRAP values of 23 vegetable juices increased after gastrointestinal digestion.

The total antioxidant activity in both the gastric and intestinal digesta was lower than in the chemical extracts. This could be due to the lower TPC as suggested by Bouayed et al.⁷². The DPPH[•] assay has been reported to underestimate AA due to the possible reactions of DPPH[•] and free radical scavengers, which are also influenced by the structural conformation of antioxidants. DPPH[•] is very sensitive to low pH, and digestion could alter the structure of the antioxidant compounds, which may affect their reactivity with the less biologically relevant

nitrogen radical formed in the DPPH[•] assay^{28, 70}. In addition, the FRAP assay is more useful to study changes in AA as related to the changes in phenolic concentration without interference because of the pH-dependent variation in the polyphenol structure^{73, 74}.

Total phenolic content

The impact of *in vitro* simulated GI digestion on the TPC is shown in Fig. 3. Mosele et al. (2016) suggested that the decrease in TPC observed after the gastric phase could be due to the instability of molecules under low pH as well as their tendency for entrapment in the pectin gel. However, Friedman & Jürgens⁷⁶ and Bermúdez-Soto et al.⁷⁷ suggested that the decrease in TPC after gastrointestinal digestion could be associated with the instability of phenolic compounds under high pH. They indicated that the small molecules formed due to hydrolysis are less stable than the larger molecules resulting in different chemical properties, bioaccessibility, bioavailability and biological activity. However, it should be noted that the Folin-Ciocalteu reagent could be reduced by other nonphenolic compounds such as sugars, amines, organic acids, proteins, and ascorbic acid, leading to an overestimation of the TPC^{69, 78}.

The greatest decrease in TPC was observed after the gastric phase followed by a smaller decrease after the intestinal phase. The decrease was approximately 80-84 % in whole fruit and 65-85 % in fresh-cut pears. The losses were lower in fresh-cut pears at day 9 (65-77 %) than at day 0 (76-85 %), as was observed for the DPPH[•] and FRAP antioxidant activity values. Pavan et al.⁷⁹ reported that the TPC of araticum and papaya extracts decreased after *in vitro* digestion. However, Attri et al.²⁸ observed an increase in the TPC of some fruit juices, and Chen et al.⁶⁸ also reported an increase in the TPC of 25 fruits after gastrointestinal digestion.

CONCLUSIONS

The effect of postharvest calcium treatment and biopreservation using the probiotic bacteria *Lactobacillus rhamnosus* GG on the enzymatic activity, nutritional quality and bioaccessibility of the total phenolic content and antioxidant activity of whole and fresh-cut pears was evaluated. The postharvest calcium treatment had no effect on the nutritional quality of fruit compared with untreated whole pears. Moreover, no synergy between calcium chloride and *Lactobacillus rhamnosus* GG was observed on the fresh-cut samples. The application of *L. rhamnosus* GG maintained the quality and the antioxidant potential of the fresh-cut product. Hence, taking into account the results obtained in this study and the reported benefits of *L. rhamnosus* GG on the microbial quality of fresh-cut pears, this treatment of fresh-cut product could be a possible alternative to the use of dairy products. Future studies should include a complementary step with Caco-2 cells in the *in vitro* gastrointestinal digestion to estimate the bioavailability of antioxidant activity and total phenolic content.

ACKNOWLEDGMENTS

The authors thank the Spanish Ministry of Science and Innovation (AGL-2012-38671). This work has been supported by the Secretaria d'Universitats i Recerca del Departament d'Economia i Coneixement (AGAUR fellowship 2017-FI-B200164 to Lorena Zudaire and 2015-FI-B100156 to M. Belén Iglesias) and the CERCA Programme of Generalitat de Catalunya. L. Plaza thanks the National Institute for Agronomic Research (INIA) for a DOC-INIA research contract. I. Aguiló-Aguayo thanks the National Programme for the Promotion of Talent and Its Employability of the 'Ministerio de Economía, Industria y Competitividad' of the Spanish Government and the European Social Fund for her Postdoctoral Senior Grant 'Ramon y Cajal' (RYC-2016-19949). The researchers also thank Daniel Martín, Silvia Villaró and Jordi Sabata for their technical support.

REFERENCES

1. Colás-Medà P, Abadías M, Alegre I, Usall J, Viñas I. Effect of ripeness stage during processing on *Listeria monocytogenes* growth on fresh-cut ‘Conference’ pears. *Food Microbiol.* 2015;49:116-122. doi:10.1016/j.fm.2015.01.019.
2. MAGRAMA. Informe del consumo de alimentación en España 2016. http://www.magrama.gob.es/imagenes/es/informeconsumoalimentacion2015_tcm7-422016.pdf. Published 2017.
3. MAGRAMA. Anuario de Estadística Avance 2016. <http://www.magrama.gob.es/es/alimentacion/temas/la-agricultura-ecologica/default.aspx>). Published 2017.
4. Rößle C, Auty MAE, Brunton N, Gormley RT, Butler F. Evaluation of fresh-cut apple slices enriched with probiotic bacteria. *Innov Food Sci Emerg Technol.* 2010;11(1):203-209. doi:10.1016/j.ifset.2009.08.016.
5. Rojas-Graü MA, Sobrino-López A, Tapia MS, Martín-Belloso O. Browning inhibition in fresh-cut ‘Fuji’ apple slices by natural antibrowning agents. *J Food Sci.* 2006;71(1):59-65.
6. Baseline A, Colantuoni F, Lass DA, Nardone G, Stasi A. Trends in EU consumers’ attitude towards fresh-cut fruit and vegetables. *Food Qual Prefer.* 2017;59:87-96. doi:10.1016/j.foodqual.2017.01.008.
7. Rojas-Graü MA, Garner E, Martín-Belloso O. The fresh-cut fruit and vegetables industry. Current situation and market trends. In: Martín-Belloso O, Soliva-Fortuny RC, eds. *Advances in Fresh-Cut Fruits and Vegetables Processing*. 1st ed. Boca Raton: CRC Press; 2010:1-13.
8. Gross KC, Wang CY, Saltveit M. *The Commercial Storage of Fruits, Vegetables, and Florist and Nursery Stocks*. (Gross KC, Wang CY, Saltveit M, eds.). Washington, DC;

2016. <http://www.ba.ars.usda.gov/hb66/>.

9. Torres E, Recasens I, Lordan J, Alegre S. Combination of strategies to supply calcium and reduce bitter pit in ‘Golden Delicious’ apples. *Sci Hortic (Amsterdam)*. 2017;217:179-188. doi:10.1016/j.scienta.2017.01.028.
10. Gago CML, Guerreiro AC, Miguel G, Panagopoulos T, da Silva MM, Antunes MDC. Effect of Calcium chloride and 1-MCP (Smartfresh™) postharvest treatment on ‘Golden Delicious’ apple cold storage physiological disorders. *Sci Hortic (Amsterdam)*. 2016;211:440-448. doi:10.1016/j.scienta.2016.09.017.
11. Holb IJ, Balla B, Vámos A, Gáll JM. Influence of preharvest calcium applications, fruit injury, and storage atmospheres on postharvest brown rot of apple. *Postharvest Biol Technol*. 2012;67:29-36. doi:10.1016/j.postharvbio.2011.12.008.
12. Kou X, Wu M, Li L, et al. Effects of CaCl₂ dipping and pullulan coating on the development of brown spot on ‘Huangguan’ pears during cold storage. *Postharvest Biol Technol*. 2015;99:63-72. doi:10.1016/j.postharvbio.2014.08.001.
13. Javed MS, Randhawa MA, Butt MS, Nawaz H. Effect of calcium lactate and modified atmosphere storage on biochemical characteristics of guava fruit. *J Food Process Preserv*. 2016;40(4):657-666. doi:10.1111/jfpp.12645.
14. Alandes L, Pérez-Munuera I, Llorca E, Quiles A, Hernando I. Use of calcium lactate to improve structure of ‘Flor de Invierno’ fresh-cut pears. *Postharvest Biol Technol*. 2009;53(3):145-151. doi:10.1016/j.postharvbio.2009.03.006.
15. Beirão-da-Costa S, Cardoso A, Martins LL, Empis J, Moldão-Martins M. The effect of calcium dips combined with mild heating of whole kiwifruit for fruit slices quality maintenance. *Food Chem*. 2008;108(1):191-197. doi:10.1016/j.foodchem.2007.10.075.
16. López-López M, Vega-Espinoza A, Ayón-Reyna L, López-Valenzuela J, Vega-García M. Combined effect of hot water dipping treatment, N-acetylcysteine and calcium on

- quality retention and enzymatic activity of fresh-cut apple. *J Food, Agric Environ.* 2013;11(2):243-248.
17. Troyo RD, Acedo AL. Optimizing the application of probiotic lactic acid bacteria for increased antimicrobial efficacy on freshcut jackfruit (*Artocarpus heterophyllus*). *Acta Hortic.* 2015;1088:419-424.
 18. FAO/WHO. Guidelines for the evaluation of probiotics in food. *Food Agric Organ United Nations World Heal Organ (Working Gr Report)*. 2002.
 19. Alegre I, Viñas I, Usall J, Anguera M, Abadias M. Microbiological and physicochemical quality of fresh-cut apple enriched with the probiotic strain *Lactobacillus rhamnosus* GG. *Food Microbiol.* 2011;28(1):59-66. doi:10.1016/j.fm.2010.08.006.
 20. Saad N, Delattre C, Urdaci M, Schmitter JM, Bressollier P. An overview of the last advances in probiotic and prebiotic field. *LWT - Food Sci Technol.* 2013;50(1):1-16. doi:10.1016/j.lwt.2012.05.014.
 21. Socol CR, Porto L, Vandenberghe DS, et al. The Potential of Probiotics : A Review. *Food Technol Biotechnol.* 2010;48(4):413-434.
 22. Iglesias MB, Viñas I, Colás-Medà P, Collazo C, Serrano JCE, Abadias M. Adhesion and invasion of *Listeria monocytogenes* and interaction with *Lactobacillus rhamnosus* GG after habituation on fresh-cut pear. *J Funct Foods.* 2017;34:453-460. doi:10.1016/j.jff.2017.05.011.
 23. Iglesias MB, Abadias M, Anguera M, Sabata J, Viñas I. Antagonistic effect of probiotic bacteria against foodborne pathogens on fresh-cut pear. *LWT - Food Sci Technol.* 2017;81:243-249. doi:10.1016/j.lwt.2017.03.057.
 24. Russo P, Peña N, de Chiara MLV, Amodio ML, Colelli G, Spano G. Probiotic lactic acid bacteria for the production of multifunctional fresh-cut cantaloupe. *Food Res Int.* 2015;77:762-772. doi:10.1016/j.foodres.2015.08.033.

25. Troyo RD, Acedo AL. Antimicrobial potential of lactic acid bacteria on freshcut pineapple (*Ananas comosus*). *Acta Hort.* 2015;1088:413-418. http://link.springer.com/chapter/10.1007/978-1-4615-2668-1_3.
26. Russo P, de Chiara MLV, Vernile A, et al. Fresh-cut pineapple as a new carrier of probiotic lactic acid bacteria. *Biomed Res Int.* 2014;2014:1-9. doi:10.1155/2014/309183, 10.1155/2014/309183.
27. Carbonell-Capella JM, Buniowska M, Esteve MJ, Frígola A. Effect of *Stevia rebaudiana* addition on bioaccessibility of bioactive compounds and antioxidant activity of beverages based on exotic fruit mixed with oat following simulated human digestion. *Food Chem.* 2015;184:122-130. doi:10.1016/j.foodchem.2015.03.095.
28. Attri S, Singh N, Singh TR, Goel G. Effect of in vitro gastric and pancreatic digestion on antioxidant potential of fruit juices. *Food Biosci.* 2017;17(July 2016):1-6. doi:10.1016/j.fbio.2016.10.003.
29. Schulz M, Biluca FC, Gonzaga LV, et al. Bioaccessibility of bioactive compounds and antioxidant potential of juçara fruits (*Euterpe edulis* Martius) subjected to *in vitro* gastrointestinal digestion. *Food Chem.* 2017. doi:10.1016/j.foodchem.2017.02.038.
30. Soriano Sancho RA, Pavan V, Pastore GM. Effect of *in vitro* digestion on bioactive compounds and antioxidant activity of common bean seed coats. *Food Res Int.* 2015;76:74-78. doi:10.1016/j.foodres.2014.11.042.
31. Zudaire L, Viñas I, Abadias M, et al. Quality and bioaccessibility of total phenols and antioxidant activity of calçots (*Allium cepa* L.) stored under controlled atmosphere conditions. *Postharvest Biol Technol.* 2017;129:118-128. doi:10.1016/j.postharvbio.2017.03.013.
32. Iglesias MB, Echeverría G, Viñas I, López ML, Abadias M. Biopreservation of fresh-cut pear using *Lactobacillus rhamnosus* GG and effect on quality and volatile compounds.

LWT - Food Sci Technol. 2018;87:581-588. doi:10.1016/j.lwt.2017.09.025.

33. Plaza L, Altisent R, Alegre I, Viñas I, Abadias M. Changes in the quality and antioxidant properties of fresh-cut melon treated with the biopreservative culture *Pseudomonas graminis* CPA-7 during refrigerated storage. *Postharvest Biol Technol.* 2016;111:25-30. doi:10.1016/j.postharvbio.2015.07.023.
34. Liu C, Ma T, Hu W, Tian M, Sun L. Effects of aqueous ozone treatments on microbial load reduction and shelf life extension of fresh-cut apple. *Int J Food Sci Technol.* 2016;51(5):1099-1109. doi:10.1111/ijfs.13078.
35. Altisent R, Plaza L, Alegre I, Viñas I, Abadias M. Comparative study of improved vs. traditional apple cultivars and their aptitude to be minimally processed as 'ready to eat' apple wedges. *LWT - Food Sci Technol.* 2014;58(2):541-549. doi:10.1016/j.lwt.2014.03.019.
36. Plaza L, Muñoz M, Ancos B de, Cano M. Effect of combined treatments of high-pressure, citric acid and sodium chloride on quality parameters of tomato puree. *Eur Food Res Technol.* 2003;216:514-519. doi:10.1007/s00217-003-0689-0.
37. Van linden V, Sila DN, Duvetter T, De Baerdemaeker J, Hendrickx M. Effect of mechanical impact-bruising on polygalacturonase and pectinmethylesterase activity and pectic cell wall components in tomato fruit. *Postharvest Biol Technol.* 2008;47(1):98-106. doi:10.1016/j.postharvbio.2007.06.006.
38. Singleton V, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *METHODS Enzimol.* 1999;299.
39. Minekus M, Alminger M, Alvito P, et al. A standardised static *in vitro* digestion method suitable for food - an international consensus. *Food Funct.* 2014;5(6):1113-1124. doi:10.1039/c3fo60702j.

40. Zhao Y, Wang C. Effect of calcium chloride in combination with salicylic acid on post-harvest freshness of apples. *Food Sci Biotechnol.* 2015;24(3):1139-1146. doi:10.1007/s10068-015-0145-5.
41. Liu H, Chen F, Lai S, Tao J, Yang H, Jiao Z. Effects of calcium treatment and low temperature storage on cell wall polysaccharide nanostructures and quality of postharvest apricot (*Prunus armeniaca*). *Food Chem.* 2017;225:87-97. doi:10.1016/j.foodchem.2017.01.008.
42. Koushesh Saba M, Sogvar OB. Combination of carboxymethyl cellulose-based coatings with calcium and ascorbic acid impacts in browning and quality of fresh-cut apples. *LWT - Food Sci Technol.* 2016;66:165-171. doi:10.1016/j.lwt.2015.10.022.
43. Sanchís E, González S, Ghidelli C, et al. Browning inhibition and microbial control in fresh-cut persimmon (*Diospyros kaki* cv. Rojo Brillante) by apple pectin-based edible coatings. *Postharvest Biol Technol.* 2016;305-310. doi:http://dx.doi.org/10.1016/j.postharvbio.2015.09.024.
44. Gomes MH, Vieira T, Fundo JF, Almeida DPF. Polyphenoloxidase activity and browning in fresh-cut 'Rocha' pear as affected by pH, phenolic substrates, and antibrowning additives. *Postharvest Biol Technol.* 2014;91:32-38. doi:10.1016/j.postharvbio.2013.12.013.
45. Soliva-Fortuny RC, Biosca-Biosca M, Grigelmo-Miguel N, Martín-Belloso O. Browning, polyphenol oxidase activity and headspace gas composition during storage of minimally processed pears using modified atmosphere packaging. *J Sci Food Agric.* 2002;82(13):1490-1496. doi:10.1002/jsfa.1209.
46. Oms-Oliu G, Aguiló-Aguayo I, Martí-Belloso O. Inhibition of browning on fresh-cut pear wedges by natural compounds. *J Food Sci.* 2006;71(3):S216-S224. <http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2621.2006.tb15644.x/abstract>.

47. Xu C, Hu W feng, Luo S, Luo H jian, Ma K, Li Y fang. Effect of composite biological preservative containing *Lactobacillus plantarum* on postharvest litchi quality. *Food Sci Biotechnol.* 2016;25(6):1639-1643. doi:10.1007/s10068-016-0252-y.
48. Konappa NM, Maria M, Uzma F, et al. Lactic acid bacteria mediated induction of defense enzymes to enhance the resistance in tomato against *Ralstonia solanacearum* causing bacterial wilt. *Sci Hortic (Amsterdam).* 2016;207:183-192. doi:10.1016/j.scienta.2016.05.029.
49. Belge B, Goulao LF, Comabella E, Graell J, Lara I. Refrigerated storage and calcium dips of ripe 'Celeste' sweet cherry fruit: Combined effects on cell wall metabolism. *Sci Hortic (Amsterdam).* 2017;219:182-190. doi:10.1016/j.scienta.2017.02.039.
50. Manganaris GA, Vasilakakis M, Diamantidis G, Mignani I. The effect of postharvest calcium application on tissue calcium concentration, quality attributes, incidence of flesh browning and cell wall physicochemical aspects of peach fruits. *Food Chem.* 2007;100(4):1385-1392. doi:10.1016/j.foodchem.2005.11.036.
51. Manganaris GA, Vasilakakis M, Diamantidis G, Mignani I. Effect of post-harvest calcium treatments on the physicochemical properties of cell wall pectin in nectarine fruit during ripening after harvest or cold storage. *J Hortic Sci Biotechnol.* 2005;80(5):611-617. doi:10.1080/14620316.2005.11511986.
52. Abeles F, Morgan P, Saltveit ME. *Ethylene in Plant Biology*. London: Academic Press; 1992.
53. Marschner H. *Mineral Nutrition of Higher Plants*. Second. London: Academic Press; 1995.
54. Fortes A, Granell A, Pezzotti M, Bouzayen M. *Molecular and Metabolic Mechanisms Associated with Fleshy Fruit Quality*. Lausanne, Switzerland: Frontiers Media SA; 2017.
55. Gomes MH, Fundo JF, Santos S, Amaro AL, Almeida DPF. Hydrogen ion concentration

- affects quality retention and modifies the effect of calcium additives on fresh-cut 'Rocha' pear. *Postharvest Biol Technol.* 2010;58(3):239-246. doi:10.1016/j.postharvbio.2010.07.004.
56. Martins EMF, Ramos AM, Martins ML, Leite Júnior BR de C. Fruit salad as a new vehicle for probiotic bacteria. *Food Sci Technol.* 2016;36(3):540-548. doi:10.1590/1678-457X.03316.
57. Cocci E, Rocculi P, Romani S, Dalla Rosa M. Changes in nutritional properties of minimally processed apples during storage. *Postharvest Biol Technol.* 2006;39(3):265-271. doi:10.1016/j.postharvbio.2005.12.001.
58. Hajilou J, Fakhimrezaei S. Effects of post-harvest calcium chloride or salicylic acid treatments on the shelf-life and quality of apricot fruit. *J Hortic Sci Biotechnol.* 2013;88(5):600-601. doi:10.1080/14620316.2013.11513012.
59. Ayón-Reyna LE, González-Robles A, Rendón-Maldonado JG, Báez-Flores ME, López-López ME, Vega-García MO. Application of a hydrothermal-calcium chloride treatment to inhibit postharvest anthracnose development in papaya. *Postharvest Biol Technol.* 2017;124:85-90. doi:10.1016/j.postharvbio.2016.10.009.
60. Rößle C, Brunton N, Gormley RT, Ross PR, Butler F. Development of potentially synbiotic fresh-cut apple slices. *J Funct Foods.* 2010;2(4):245-254. doi:10.1016/j.jff.2010.09.001.
61. Aguayo E, Requejo-Jackman C, Stanley R, Woolf A. Hot water treatment in combination with calcium ascorbate dips increases bioactive compounds and helps to maintain fresh-cut apple quality. *Postharvest Biol Technol.* 2015;110:158-165. doi:10.1016/j.postharvbio.2015.07.001.
62. Bico SLS, Raposo MFJ, Morais RMSC, Morais AMMB. Combined effects of chemical dip and/or carrageenan coating and/or controlled atmosphere on quality of fresh-cut

- banana. *Food Control*. 2009;20(5):508-514. doi:10.1016/j.foodcont.2008.07.017.
63. Aguayo E, Requejo-Jackman C, Stanley R, Woolf A. Effects of calcium ascorbate treatments and storage atmosphere on antioxidant activity and quality of fresh-cut apple slices. *Postharvest Biol Technol*. 2010;57(1):52-60. doi:10.1016/j.postharvbio.2010.03.001.
64. Hossain MI, Sadekuzzaman M, Ha S-D. Probiotics as potential alternative biocontrol agents in the agriculture and food industries: A review. *Food Res Int*. 2017;100:63-73. doi:10.1016/j.foodres.2017.07.077.
65. Moreira RM, Martins ML, Leite Júnior BR de C, et al. Development of a juçara and Ubá mango juice mixture with added *Lactobacillus rhamnosus* GG processed by high pressure. *LWT - Food Sci Technol*. 2017;77:259-268. doi:10.1016/j.lwt.2016.11.049.
66. De Oliveira PM, Leite Junior BR, Martins ML, Furtado Martins EM, Ramos AM. Minimally processed yellow melon enriched with probiotic bacteria. *Semin Agrar*. 2014;35(5):2415-2426. doi:10.5433/1679-0359.2014v35n5p2415.
67. Alegre I, Viñas I, Usall J, Anguera M, Abadias M. Microbiological and physicochemical quality of fresh-cut apple enriched with the probiotic strain *Lactobacillus rhamnosus* GG. *Food Microbiol*. 2011;28(1):59-66. doi:10.1016/j.fm.2010.08.006.
68. Chen G-L, Chen S-G, Zhao Y-Y, Luo C-X, Li J, Gao Y-Q. Total phenolic contents of 33 fruits and their antioxidant capacities before and after *in vitro* digestion. *Ind Crops Prod*. 2014;57:150-157. doi:10.1016/j.indcrop.2014.03.018.
69. Rodríguez-Roque MJ, Rojas-Graü MA, Elez-Martínez P, Martín-Belloso O. Changes in vitamin C, phenolic, and carotenoid profiles throughout *in vitro* gastrointestinal digestion of a blended fruit juice. *J Agric Food Chem*. 2013;61(8):1859-1867. doi:10.1021/jf3044204.
70. Wootton-Beard PC, Moran A, Ryan L. Stability of the total antioxidant capacity and

- total polyphenol content of 23 commercially available vegetable juices before and after *in vitro* digestion measured by FRAP, DPPH, ABTS and Folin-Ciocalteu methods. *Food Res Int.* 2011;44(1):217-224. doi:10.1016/j.foodres.2010.10.033.
71. Stanisavljevic N, Samardzic J, Jankovic T, et al. Antioxidant and antiproliferative activity of chokeberry juice phenolics during *in vitro* simulated digestion in the presence of food matrix. *Food Chem.* 2015;175:516-522. doi:10.1016/j.foodchem.2014.12.009.
72. Bouayed J, Hoffmann L, Bohn T. Total phenolics, flavonoids, anthocyanins and antioxidant activity following simulated gastro-intestinal digestion and dialysis of apple varieties: Bioaccessibility and potential uptake. *Food Chem.* 2011;128(1):14-21. doi:10.1016/j.foodchem.2011.02.052.
73. Tagliazucchi D, Verzelloni E, Bertolini D, Conte A. In vitro bio-accessibility and antioxidant activity of grape polyphenols. *Food Chem.* 2010;120(2):599-606. doi:10.1016/j.foodchem.2009.10.030.
74. Tagliazucchi D, Verzelloni E, Conte A. The first tract of alimentary canal as an extractor. release of phytochemicals from solid food matrices during simulated digestion. *J Food Biochem.* 2012;36(5):555-568. doi:10.1111/j.1745-4514.2011.00569.x.
75. Mosele JI, Macià A, Romero M-P, Motilva M-J. Stability and metabolism of Arbutus unedo bioactive compounds (phenolics and antioxidants) under in vitro digestion and colonic fermentation. *Food Chem.* 2016;201:120-130. doi:10.1016/j.foodchem.2016.01.076.
76. Friedman M, Jürgens HS. Effect of pH on the stability of plant phenolic compounds. *J Agric Food Chem.* 2000;48(6):2101-2110. doi:10.1021/jf990489j.
77. Bermúdez-Soto M-J, Tomás-Barberán F-A, García-Conesa M-T. Stability of polyphenols in chokeberry (*Aronia melanocarpa*) subjected to in vitro gastric and pancreatic digestion. *Food Chem.* 2007;102(3):865-874.

doi:10.1016/j.foodchem.2006.06.025.

78. Bouayed J, Deußer H, Hoffmann L, Bohn T. Bioaccessible and dialysable polyphenols in selected apple varieties following *in vitro* digestion vs. their native patterns. *Food Chem.* 2012;131(4):1466-1472. doi:10.1016/j.foodchem.2011.10.030.
79. Pavan V, Sancho RAS, Pastore GM. The effect of *in vitro* digestion on the antioxidant activity of fruit extracts (*Carica papaya*, *Artocarpus heterophyllus* and *Annona marcgravii*). *LWT - Food Sci Technol.* 2014;59(2P2):1247-1251. doi:10.1016/j.lwt.2014.05.040.

FIGURE CAPTIONS

Fig. 1. Effect of postharvest calcium chloride treatment and biopreservation using *Lactobacillus rhamnosus* GG [NoCa/NoLGG (●), NoCa/LGG (▼), Ca/NoLGG (○) and Ca/LGG (Δ)] on the overall visual quality of fresh-cut pears during a 9-day storage period at 4 °C. The error bars represent the standard deviation of the three replicates with a 95 % confidence interval.

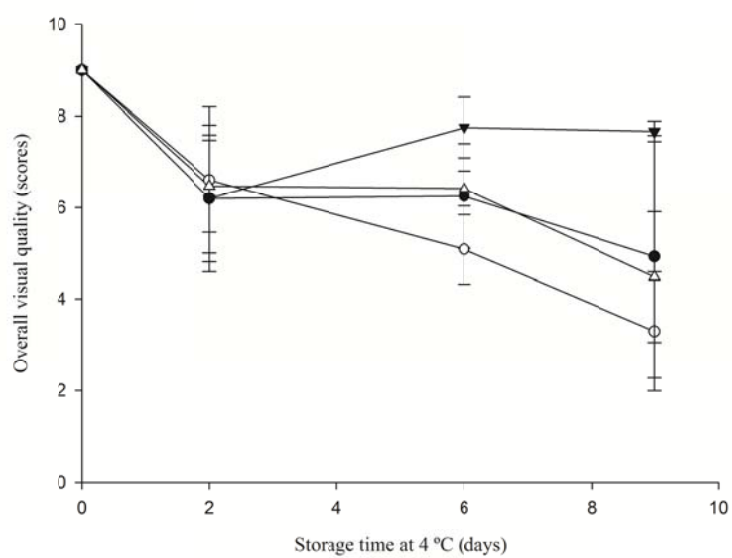
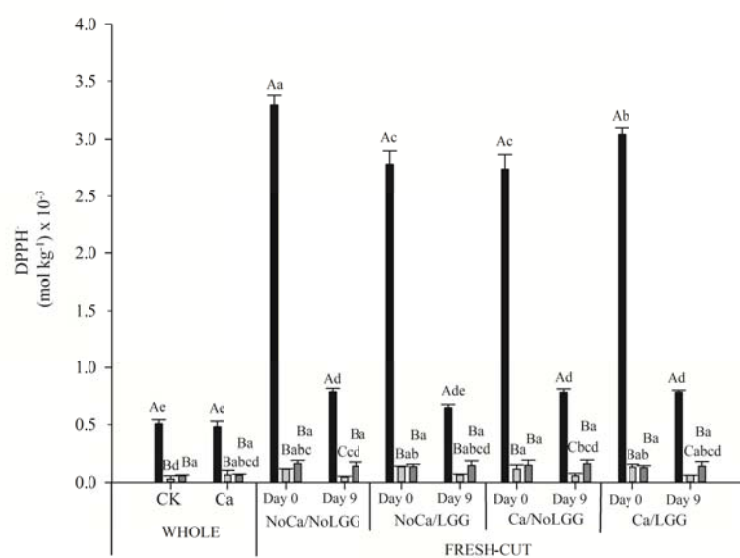


Fig. 2. Antioxidant activity (A: DPPH assay, B: FRAP assay) of whole and fresh-cut pears after *in vitro* simulated gastrointestinal (GI) digestion. Whole pears were harvested and treated (Ca) or not treated (CK) with CaCl_2 after harvest and stored at 0-0.5 °C for 8 months. After this storage period, they were processed and treated or not treated with 10^8 CFU mL^{-1} of *L. rhamnosus* GG. The *in vitro* simulated gastrointestinal digestion was performed with whole (day 0) and fresh-cut pears (day 0 and 9). Antioxidant activity was measured in non-treated (black bars), gastric digested (light grey bars) and gastrointestinal digested (dark grey bars) samples. Values are expressed as the mean \pm standard deviation. Capital letters indicate significant differences ($P < 0.05$) between the phases of *in vitro* simulated gastrointestinal (GI) digestion. Lower case letters indicate significant differences ($P < 0.05$) between storage treatments at same storage time and digestion phase.



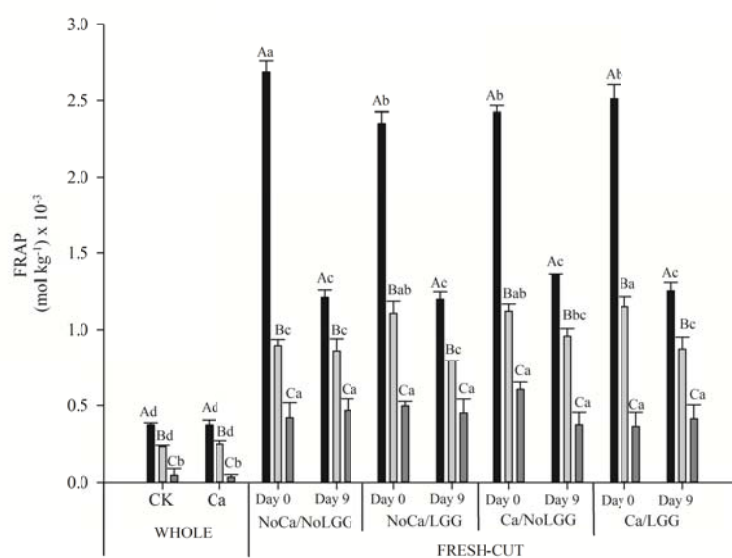
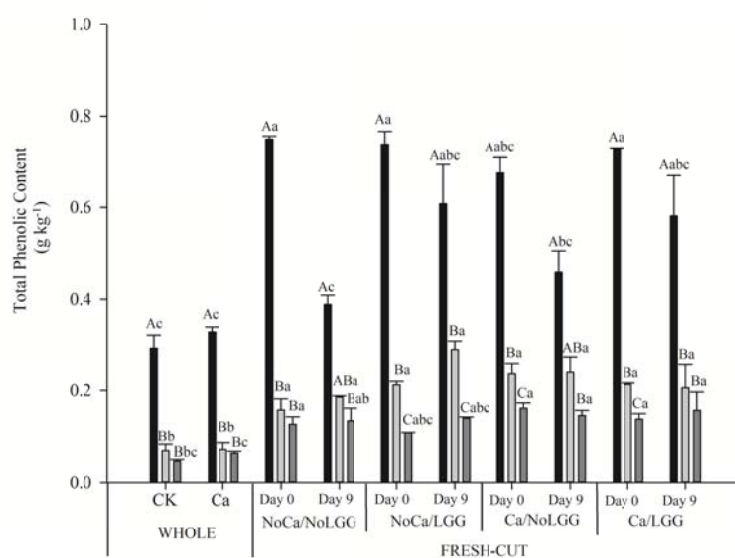


Fig. 3. Total phenolic content of whole and fresh-cut pears after *in vitro* simulated gastrointestinal (GI) digestion. Whole pears were harvested and treated (Ca) or not treated (CK) with CaCl_2 after harvest and stored at 0-0.5 °C for 8 months. After this storage period, they were processed and treated or not treated with 10^8 CFU mL^{-1} of *L. rhamnosus* GG. *In vitro* simulated gastrointestinal digestion was performed with whole (day 0) and fresh-cut pears (day 0 and 9). Total phenolic content was measured in non-treated (black bars), gastric digested (light grey bars) and gastrointestinal digested (dark grey bars) samples. Values are expressed as the mean \pm standard deviation. Capital letters indicate significant differences ($P < 0.05$) between the phases of *in vitro* simulated gastrointestinal (GI) digestion. Lower case letters indicate significant differences ($P < 0.05$) between storage treatments at same storage time and digestion phase.



TABLES

Table 1. Quality parameters of whole pears after 8 months of storage at 0-0.5 °C under controlled atmospheric conditions (2 % O₂ and 1 % CO₂) and 85 % RH.

Parameters	No Calcium	Calcium (1 %)
Acidity (g malic acid L ⁻¹)	2.20 ± 0.12 a	1.73 ± 0.18 b
Soluble Solid Content (%)	14.13 ± 0.88 a	13.50 ± 0.38 a
Firmness (N)	44.12 ± 7.61 a	40.50 ± 7.92 a
<i>L</i> *	64.49 ± 4.72 a	63.37 ± 5.44 a
<i>a</i> *	-13.40 ± 2.46 a	-12.47 ± 2.71 a
<i>b</i> *	38.77 ± 3.51 a	37.79 ± 3.60 a
PPO (ΔOD s ⁻¹ kg ⁻¹) ^a	270.93 ± 28.34 b	447.30 ± 21.80 a
POD (ΔOD s ⁻¹ kg ⁻¹)	170.12 ± 20.72 b	234.96 ± 14.99 a
PME (mol s ⁻¹ kg ⁻¹) x 10 ⁻⁵	1.02 ± 0.05 a	0.85 ± 0.10 a
PG (mol s ⁻¹ kg ⁻¹) x 10 ⁻⁵	1.70 ± 0.34 b	2.34 ± 0.15 a
Vitamin C (g kg ⁻¹) x 10 ⁻²	0.23 ± 0.04 a	0.23 ± 0.04 a
Phenols (g kg ⁻¹)	0.29 ± 0.03 a	0.33 ± 0.01 a
Antioxidant activity		
DPPH (mol kg ⁻¹) x 10 ⁻³	0.51 ± 0.04 a	0.48 ± 0.05 a
FRAP (mol kg ⁻¹) x 10 ⁻³	0.37 ± 0.01 a	0.37 ± 0.03 a
Calcium (g kg ⁻¹)	0.58 ± 0.00 a	0.58 ± 0.01 a

Values are the mean of independent determinations ± standard deviation. Different letters in the same row indicate significant differences (*P*<0.05). ^aOD, optical densitometry.

Table 2. Physical parameters and enzymatic activity of fresh-cut pears during storage at 4 °C.

Treatments	Storage time (days)	Firmness (N)	Browning Index	PPO ($\Delta\text{OD s}^{-1} \text{ kg}^{-1}$) ^a	POD ($\Delta\text{OD s}^{-1} \text{ kg}^{-1}$)	PME ($\text{mol s}^{-1} \text{ kg}^{-1}$) $\times 10^{-5}$	PG ($\text{mol s}^{-1} \text{ kg}^{-1}$) $\times 10^{-5}$
NO CALCIUM							
Control	0	10.18 \pm 2.02 Aa	17.02 \pm 3.71 Aa	728.93 \pm 64.17 Aa	114.99 \pm 23.46 Bb	1.11 \pm 0.19 Aa	1.94 \pm 0.20 Bb
	2	15.95 \pm 3.17 Aa	18.43 \pm 5.12 Aa	315.11 \pm 59.06 Bc	292.04 \pm 31.53 Ab	1.75 \pm 0.40 Aa	2.58 \pm 0.40 ABa
	6	13.51 \pm 1.98 Aa	17.66 \pm 3.60 Ab	322.69 \pm 24.47 Bbc	260.81 \pm 28.84 Aab	1.88 \pm 0.32 Aa	3.01 \pm 0.46 Aa
	9	15.13 \pm 3.42 Aa	19.30 \pm 3.80 Ab	294.82 \pm 35.68 Bc	253.23 \pm 28.46 Aa	1.31 \pm 0.27 Aa	1.86 \pm 0.36 Ba
LGG	0	10.06 \pm 4.25 Aa	17.83 \pm 4.94 Aa	764.01 \pm 14.07 Aa	213.42 \pm 6.92 Ba	0.71 \pm 0.15 Bb	3.96 \pm 0.40 Aa
	2	13.87 \pm 2.70 Aab	15.86 \pm 4.08 Aa	611.63 \pm 18.75 Ba	367.25 \pm 29.87 Aa	1.65 \pm 0.13 Aa	2.07 \pm 0.15 Bab
	6	13.21 \pm 2.18 Aa	17.61 \pm 4.80 Ab	394.10 \pm 87.23 Cab	431.61 \pm 47.93 Aa	1.87 \pm 0.28 Aa	1.44 \pm 0.40 Bb
	9	14.27 \pm 2.61 Aa	17.86 \pm 3.96 Ab	401.27 \pm 11.93 Cb	335.53 \pm 74.83 ABa	2.01 \pm 0.28 Aa	1.72 \pm 0.20 Ba
CALCIUM							
Control	0	10.44 \pm 1.75 Aa	15.68 \pm 3.48 Ca	354.50 \pm 19.89 Bb	189.93 \pm 22.92 Ba	0.79 \pm 0.11 Bab	1.77 \pm 0.24 Ab
	2	13.99 \pm 2.04 Aab	17.86 \pm 4.64 BCa	445.49 \pm 51.06 Ab	301.74 \pm 25.49 Aab	2.32 \pm 0.61 Aa	2.29 \pm 0.26 Aab
	6	12.63 \pm 1.87 Aa	21.94 \pm 7.62 ABa	256.44 \pm 22.19 Cc	248.77 \pm 25.58 ABb	1.96 \pm 0.13 Aa	1.58 \pm 0.38 Ab
	9	12.35 \pm 4.02 Aa	24.11 \pm 7.55 Aa	507.63 \pm 3.38 Aa	280.01 \pm 36.85 Aa	1.53 \pm 0.19 ABa	1.67 \pm 0.46 Aa
LGG	0	11.67 \pm 2.90 Aa	19.47 \pm 6.00 Aa	336.12 \pm 54.85 Bb	203.72 \pm 37.76 Ba	0.73 \pm 0.05 Cb	1.83 \pm 0.16 Ab
	2	12.66 \pm 1.45 Ab	18.14 \pm 4.66 Aa	405.91 \pm 18.93 Bbc	286.01 \pm 21.64 ABb	1.35 \pm 0.28 BCa	1.65 \pm 0.23 Ab
	6	13.40 \pm 2.57 Aa	19.37 \pm 5.33 Aab	511.62 \pm 18.83 Aa	409.98 \pm 81.16 Aa	2.19 \pm 0.40 Aa	2.09 \pm 0.26 Aab
	9	12.76 \pm 1.07 Aa	19.88 \pm 6.28 Ab	521.77 \pm 29.58 Aa	242.58 \pm 41.43 Ba	1.54 \pm 0.32 ABa	1.56 \pm 0.39 Aa

Values are the mean of independent determinations \pm standard deviation. Different capital letters in the same treatment indicate significant differences between days ($P < 0.05$). Different lower-case letters for the same storage day indicate significant differences ($P < 0.05$) between treatments. ^aOD, optical density.

Table 3. Vitamin C, phenolic, and calcium content and antioxidant activity of fresh-cut pears during storage at 4 °C.

Treatments	Storage time (days)	Vitamin C (g kg ⁻¹) x 10 ⁻²	Phenols (g kg ⁻¹)	DPPH [•] (mol kg ⁻¹) x 10 ⁻³	FRAP (mol kg ⁻¹) x 10 ⁻³	Calcium (g kg ⁻¹)
NO CALCIUM						
Control	0	10.30 ± 0.69 Ab	0.75 ± 0.01 ABa	3.29 ± 0.09 Aa	2.69 ± 0.07 Aa	1.94 ± 0.04 Cb
	2	4.18 ± 0.32 Bab	0.84 ± 0.13 Aa	1.00 ± 0.04 Ba	1.36 ± 0.05 Ba	2.13 ± 0.02 Aa
	6	1.31 ± 0.12 Cb	0.63 ± 0.14 ABa	0.73 ± 0.06 Cbc	1.10 ± 0.06 Ca	1.81 ± 0.01 Dc
	9	0.99 ± 0.07 Cb	0.39 ± 0.02 Ba	0.79 ± 0.03 Ca	1.21 ± 0.05 BCb	2.00 ± 0.02 Ba
LGG	0	8.89 ± 0.04 Ab	0.74 ± 0.03 ABa	2.78 ± 0.12 Abc	2.35 ± 0.08 Ab	1.66 ± 0.01 Cc
	2	4.50 ± 0.15 Ba	0.80 ± 0.05 Aa	0.91 ± 0.06 Ba	1.19 ± 0.01 Bb	1.88 ± 0.01 Bbc
	6	1.40 ± 0.09 Cb	0.63 ± 0.08 ABa	0.64 ± 0.04 Cc	1.16 ± 0.08 Ba	2.00 ± 0.04 Aa
	9	1.31 ± 0.17 Ca	0.61 ± 0.09 Ba	0.65 ± 0.03 Cb	1.20 ± 0.05 Bb	1.96 ± 0.00 Aa
CALCIUM						
Control	0	13.20 ± 0.68 Aa	0.68 ± 0.03 Ba	2.74 ± 0.13 Ac	2.42 ± 0.04 Ab	2.18 ± 0.00 Aa
	2	4.22 ± 0.41 Bab	0.88 ± 0.03 Aa	0.91 ± 0.05 Ba	1.14 ± 0.07 Bb	2.01 ± 0.02 Bb
	6	1.38 ± 0.04 Cb	0.64 ± 0.13 BCa	0.84 ± 0.03 Ba	1.16 ± 0.16 Ba	1.95 ± 0.01 Cb
	9	1.37 ± 0.07 Ca	0.46 ± 0.05 Ca	0.78 ± 0.03 Ba	1.35 ± 0.01 Ba	1.97 ± 0.01 BCa
LGG	0	10.10 ± 0.74 Ab	0.73 ± 0.00 ABa	3.04 ± 0.06 Aab	2.51 ± 0.09 Aab	2.03 ± 0.02 Ab
	2	3.71 ± 0.22 Bb	0.88 ± 0.07 Aa	0.88 ± 0.11 Ba	1.33 ± 0.03 Ba	1.77 ± 0.16 BCc
	6	1.66 ± 0.10 Ca	0.70 ± 0.07 ABa	0.78 ± 0.03 Bab	1.35 ± 0.04 Ba	1.80 ± 0.00 Bc
	9	1.11 ± 0.05 Cab	0.58 ± 0.09 Ba	0.78 ± 0.02 Ba	1.26 ± 0.05 Bab	1.75 ± 0.00 Cb

Values are the mean of independent determinations ± standard deviation. Different capital letters for the same treatment indicate significant differences between days (P>0.05). Different lower-case letters for the same storage day indicate significant differences (P>0.05) between treatments.